

ON THE CONTRIBUTION OF DYNEIN-LIKE ACTIVITY TO TWISTING IN A THREE-DIMENSIONAL SLIDING FILAMENT MODEL

MICHAEL HINES AND JACOB J. BLUM

Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT It has been shown (Hines, M., and J. J. Blum, *Biophys. J.*, 1984, 46:559–565) that passive moment-bearing links do not contribute appreciable twist resistance to an axoneme nor do they cause appreciable twisting in response to internal shear forces. We now examine the contribution of active moment-bearing links such as dynein arms to the generation of twist within an axoneme. The dynein model used causes distal sliding of the adjacent doublet by a force dependent on the angle of attachment of the arms. Attachment of the arms occurs at a specified angle relative to the angle of minimum potential energy. The steady state shape consistent with the forces applied by the attached dyneins is computed. It is shown that the twist generated in an active region is counterclockwise as viewed from tip to base and therefore accumulates at the end of the axoneme. For realistic forces and twist resistances, cumulative twist should not exceed a few degrees.

INTRODUCTION

This is the third of a series of papers in which we investigate the generation of axonemal twist within the framework of a strict sliding filament model, i.e., the relative location of structural elements within any cross section of the axoneme is fixed except that doublets may slide. Specification of three-dimensional shape can therefore be characterized by the relative orientation of adjacent cross sections as one moves along the length of the axoneme (Hines and Blum, 1983). Relative rotation between two adjacent cross sections about the longitudinal (z) axis is twist.

Twist resistance of the whole axoneme is the sum of the contributions of the twist resistances of the microtubules and of links connecting them. Because links whose angle is not fixed relative to the doublets are not distorted by twist, such links do not contribute twist resistance (Hines and Blum, 1983). In the first paper we showed that external forces may cause appreciable twist, largely because external forces generally can have long moment arms perpendicular to a cross section, but partly because internal twist resistance is small.

In the second paper of this series (Hines and Blum, 1984), it was shown that moment-bearing links, which are distorted by twist, contribute negligibly to twist resistance. The dominant effect of such moment-bearing links is to couple sliding to twist. An example is the contribution of 5–6 bridges to axonemal twist caused by internal forces tending to shear those passive bridges. This contribution becomes significant only when the bridges are very stiff and there is a large shear force acting to cause the sliding, a

combination that presumably does not occur normally in flagellar beating. This raises the question, however, whether active moment-bearing links (e.g., dynein arms) that could produce large amounts of sliding might produce appreciable twist as a result of the accumulation of small increments of twist per force generating cycle. Here we address this issue and show that all active dyneins tend to generate counterclockwise twist that is cumulative. If the active dyneins are located on opposite sides of the axoneme but at the same cross section, no net sliding will occur but twist could be noticeable. For dyneins localized at differing arc lengths, so that sliding will occur, twist is cumulative but negligible in magnitude.

REPRESENTATION OF ACTIVE MOMENT-BEARING LINKS

In a previous paper (Hines and Blum, 1984), it was shown that for a fixed moment-bearing link originating on filament $j = i - 1$ and attached to a binding site on filament i , the angle, ϵ_{ij} , that the link makes to L_{ij} is

$$\epsilon_{ij} = \frac{\gamma \cdot \mathbf{T} \times \mathbf{L}_{ij} + \mathbf{L}_{ij} \cdot \boldsymbol{\kappa} \times \mathbf{L}_i}{L_{ij}}, \quad (1)$$

where \mathbf{L}_i is a vector of magnitude L in the cross section of the axoneme from the neutral axis to the binding site on the i th filament, $\mathbf{L}_{ij} = \mathbf{L}_i - \mathbf{L}_j$, \mathbf{T} is the tangent to the neutral axis at the cross section, γ is the shear angle of the axoneme at arc length s (our generalized coordinate). The shear angle, γ , is related to sliding via the relation $u_{ij} = \gamma \cdot \mathbf{T} \times \mathbf{L}_{ij}$. In Eq. (1), ϵ_{ij} is assumed to be small. This equation must be modified to describe dynein arms that attach at an

angle ϵ_a (irrespective of γ) and, while attached, have a potential energy density (units: piconewton microns per micron) given by

$$U_{ij} = \frac{k}{2}(\epsilon_{ij} - \epsilon_a)^2, \quad (2)$$

where ϵ_a is the angle of minimal energy and the force constant, k , is in units of piconewtons per radian per radian.

The body coordinate system we use (Hines and Blum, 1983, 1984) is oriented such that the z direction is tangent to the neutral axis, and the x direction is directed from the neutral axis toward doublet number 1 in the cross section under consideration. The direction of y is towards doublets 2 and 3, which makes the coordinate system right handed. Without loss of generality, we shall consider dyneins that cause bending only in the xz plane, i.e., the plane perpendicular to the 5–6 bridge. If a dynein arm attaches at t^* at angle ϵ_a , then for $t > t^*$ until the dynein detaches we model its potential energy by

$$U_y = \frac{k}{2}(-\gamma_y + L\kappa_z - \epsilon_y^*)^2 \quad (3)$$

$$U_{-y} = \frac{k}{2}(\gamma_y + L\kappa_z - \epsilon_{-y}^*)^2, \quad (4)$$

where U_y and U_{-y} are the potential energies of dyneins on the 2, 3 side of the axoneme and the 7, 8 side, respectively, and $\epsilon_y^* = -\gamma_y(t^*) + L\kappa_z(t^*) - (\epsilon_a - \epsilon_d)$ and $\epsilon_{-y}^* = \gamma_y(t^*) + L\kappa_z(t^*) - (\epsilon_a - \epsilon_d)$. Notice that the twisting moment of dyneins active on the 2, 3 doublet pair is

$$M_z = -\frac{\partial U_y}{\partial \kappa_z} = -\kappa L(-\gamma_y + L\kappa_z - \epsilon_y^*). \quad (5)$$

Since dyneins move the B-subfilament distally (Sale and Satir, 1977) the sliding, u_{32} is in the positive direction and, hence, dyneins on the 2–3 side of the axoneme cause the shear angle, γ_y , to move in a negative direction. Since this motion reduces the energy of the attached dynein arm, the quantity $(-\gamma_y - \epsilon_y^*)$ in Eq. 3 must move towards zero and therefore must be negative. From Eq. 5, a 2–3 dynein arm generates positive twisting moment resulting in a counterclockwise twist as viewed from the tip. Similarly, a 7–8 dynein arm causes γ_y to move in a positive direction and also cause counterclockwise twist. (The twist resistance term $L\kappa_z$ in Eq. 5 is very small and only slightly decreases the amount of twist obtained.) Thus the consequence of a distally directed dynein force on the B-subfilament is a counterclockwise twist (viewed from the tip) regardless of the location of the attached dynein arm.

RESULTS

Computer simulations of the effect of active moment-bearing links (i.e., dyneins) were carried out using the computer program described in Hines and Blum (1984)

with the dynein shear moments directed along the y axis, i.e., perpendicular to the moments generated by the passive 5–6 bridges had they been present.

In Figs. 1 and 2, the following parameter values were used: axonemal length, 40 μm , divided for purpose of computation into 30 segments; $L = 100$ nm; the passive resistance to shear, $E_s = 10$ pN; the bending resistances $E_{b,x} = E_{b,y} = 300$ pN μm^2 , and the twist resistance $E_{b,z} = 500$ pN μm^2 (Ishijima and Hiramoto, 1982; discussed in Hines and Blum, 1983). The force constant, k , in Eq. 2 was taken as 100 pN/rad², consistent with a force constant per dynein arm of 1.6 pN/nm. This value is consistent with motion of the arms over a distance of about 100 nm per ATP hydrolysis event (see, e.g., Brokaw, 1976). The shear resistance, $E_{s,6}$, of the 5–6 bridge (which resists shear angle only in the x direction) is taken as zero in all computations. The magnitude of $\epsilon_a - \epsilon_d$ was taken as 0.5 rad (Satir et al., 1981). For simplicity we treat the 2–3 and 7–8 dyneins as lying in the xz plane.

Fig. 1 shows the accumulated changes in κ_y and κ_z caused by the repeated cycling of 7–8 dynein arms located from 13 to 27 μm along the axoneme. Since we assume that dynein force components tending to shear the $E_{s,6}$ bridges are zero, $\kappa_x = 0$ and is not shown. The dynein cycling procedure is as follows. The flagellum is initially straight and dyneins at the specified locations become active and attach at ϵ_a , 0.5 rad from their angles of minimum energy. The resulting steady state equilibrium shape is computed. (Note that although ϵ_d is the angle of minimum energy of the arms, this does not imply that detachment will occur at this angle.) Keeping the resultant shape fixed we now detach all the dyneins in the specified region and reattach them at ϵ_a and calculate the new equilibrium shape. Since we are not concerned here with bend propagation we have allowed dynein cycling to occur only at specified arc lengths. Fig. 2 shows that after 10 such cycles there is hardly any further increment in κ_y or κ_z . It can be seen that these dyneins have sufficient strength so that for the first few cycles they produce close to 0.5 rad of sliding per cycle. It is only in the later cycles that the opposing bend and shear resistances are such that equilibrium occurs when the dynein moves

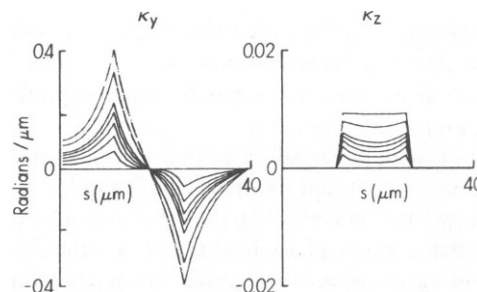


FIGURE 1 Cumulative effects of dynein cycling on bending and twist. Dynein arms originating on doublet 7 and acting to move doublet 8 distally were active in segments 10–20. The curvatures κ_y and κ_z are shown for the first 5 dynein cycles and then the 10th and 20th cycles.

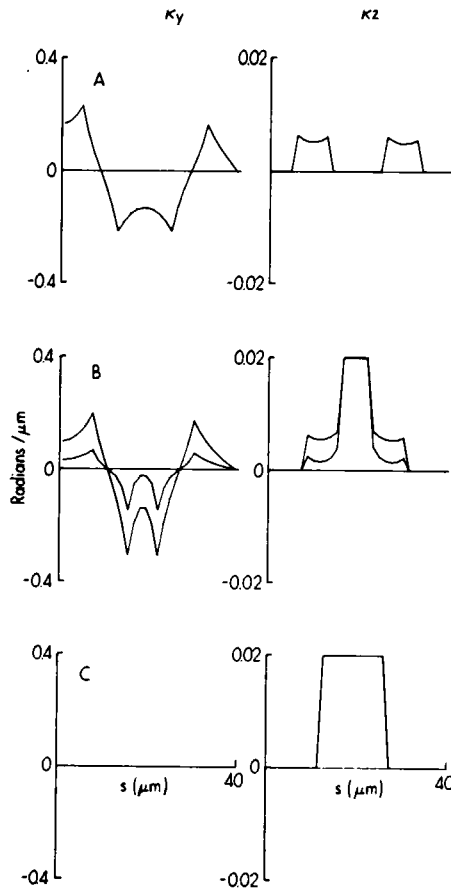


FIGURE 2 Effects of location of opposing dynein regions of activity on curvature and twist. In these three simulations dynein arms were simultaneously active on both sides of the axoneme, i.e., on doublets 2, 3 and 7, 8. In *A*, the 7, 8 active region was from segments 5 to 10 and the 2, 3 active region was from segments 20 to 25. In *B*, the active regions were segments 7 to 17 and 13 to 23. In *C*, both active regions were located in segments 10 to 20. *A* shows the steady state curvatures after four cycles of attachment and detachment; *B* after one and four cycles; *C* after one cycle (κ_z increases negligibly after further cycles).

only partially down its energy curve. After about five cycles of such "ratcheting" the flagellum assumes an S-shaped curve with a total shear typical of that seen in real flagella. An analogous simulation but with the dynein arms active on the 2-3 side yielded, as expected, the opposite curvature in the y direction and the same counterclockwise twist (not shown).

When dynein arms are active on opposite sides of an axoneme and are separated by some arc length, the shape of a bending wave is produced. If the active dynein regions are sufficiently separated, the twist produced in one region is not affected by the activity in the other region (Fig. 2 *A*). As the active regions approach one another, a small increase in twist occurs in the proximate portion of each active region (not shown). When the active regions overlap by even one segment, twisting attains its maximal value ($\approx 1^\circ/\mu\text{m}$) in the overlap zone. Fig. 2 *B* shows the curvature obtained when the overlap is five segments. In an

overlap region, full twist is essentially achieved after one cycle. Finally, when the active regions are directly opposite one another, no bending occurs ($\kappa_y = 0$) and twisting is constant (and essentially maximal) throughout the entire overlap region. Because the twist is so small, the presence of 5, 6 bridges (i.e., passive moment-bearing links that resist shear moments in the x direction) has negligible effect on either the twist or the bending in the yz plane (simulations not shown).

DISCUSSION

To extend our previous study of twist production by passive moment-bearing links to dynein arms, we have used a representation of dynein cycling that is unrealistic in its details but maintains the essential features relevant to an analysis of twist. The detachment-reattachment step is considered to take place in unison irrespective of the positions of the dynein arms on their free-energy curves and each arm reattaches at the same point along the free-energy curve at angle ϵ_a . This representation permits analysis of the cumulative effects of the ratcheting of the arms on twist while ignoring the statistical aspects of the attachment-detachment cycle. The analysis shows that the twisting moment produced by a dynein shear force is proportional to L (roughly the radius of the axoneme) and is therefore small relative to the bending moment (Eq. 5) and that dyneins cause counterclockwise twist when viewed from tip to base. With our choice of parameters, the maximal twisting moment that is likely to accumulate is $\sim 0.01 \text{ rad}/\mu\text{m}$. For a region of dynein arm activity of, say, $5 \mu\text{m}$ length, the amount of twist developed across the active region would be $< 3^\circ$.

Twisting is a local effect of dynein activity. Unless two active regions are very close together, twisting curvature in one region does not affect twisting curvature in the other region (Fig. 2 *A*). When active regions overlap, there is an abrupt increase in twist to a level that is almost independent of the number of attachment-detachment cycles in the overlap region (Figs. 2 *B, C*). In regions of overlap the moment equilibrium equations can be solved analytically. Since the potential throughout the flagellum is independent of γ_z , the Euler equation for the twisting coordinate reduces to

$$\frac{d}{ds} \left(\frac{\partial U}{\partial \kappa_z} \right) = 0. \quad (6)$$

Since $\partial U / \partial \kappa_z = 0$ at the distal end of an axoneme, the constant of integration is zero and we have $\partial U / \partial \kappa_z = 0$.

The potential energy is

$$U = U_y + U_{-y} + \frac{E_b}{2} \kappa^2 + \frac{E_s}{2} \gamma^2, \quad (7)$$

where U_y and U_{-y} are given in Eqs. 3 and 4 and E_b and E_s are the bend and shear resistances of the axoneme, respec-

tively. From Eq. 6 one obtains

$$\kappa_z = \frac{2kL(\epsilon_d - \epsilon_a) + 2kL^2\kappa_z(t^*)}{E_{b_n} + 2kL^2}. \quad (8)$$

Notice that the dyneins add a very small (proportional to L^2) increment to the twist resistance, E_{b_n} . After the first cycle of attachment, $\kappa_z = 2kL(\epsilon_d - \epsilon_a)/E_{b_n}$, where $\kappa_z(t^* = 0) = 0$ for a straight flagellum, and we are neglecting the $2kL^2$ term in the denominator. Subsequent cycles increase κ_z by the amount $2kL^2/E_{b_n}$ times the curvature, $\kappa_z(t^*)$, at the end of the previous cycle. With our choice of parameters, this factor is 0.004. Thus the twist in regions of overlap reaches its maximal value after one cycle.

In this series of papers, we have demonstrated that for a strict sliding filament model (where interdoublet distances remain constant and maintain a fixed orientation with respect to the central pair) no twist is generated by pinned links. The amount of twist generated by passive moment-bearing links (e.g., 5–6 bridges) is also negligible. Active moment-bearing links, such as dynein arms, may produce small amounts ($\approx 2^\circ$ to 3°) of cumulative twist in their active region. The total twist at the distal end of the axoneme is the sum of that contributed by each active region and will be in the counterclockwise direction as viewed from tip to base.

Holwill et al. (1979) have analyzed three-dimensional bending and twisting of cilia. Their parameter τ and our twist, κ_z , are the same for planar shapes. If the flagellum bends in three dimensions, however, τ includes any twist of the neutral surface that may have occurred, whereas κ_z is independent of the angle of the neutral surface relative to the central pair. They found that the pattern of tip displacement observed in the effective and recovery pointing strokes of the cilia of *Elliptio* could be explained in three ways: a κ_z of 10 to $20^\circ/\mu\text{m}$; a planar bend whose neutral surface did not contain the central pair; or a three-dimensional shape in which the tangent at the distal end of the cilium in the recovery pointing position was at a suitable angle relative to the plane of the bend during the

effective stroke. In the latter case, i.e., three-dimensional bending, twist due to external (e.g., viscous) forces may also have occurred. Thus our conclusions that κ_z will be small as a consequence of dynein activity is not in conflict with the observed tip displacement patterns.

Since negligible twisting moments will arise due to internal forces, then, in the absence of external forces twist will not occur. Under those circumstances, three-dimensional flagellar motion can be separated into two planar problems where the motion is projected onto two perpendicular planes. A two-filament model constrained to bend in a plane is thus an appropriate simplification of a three-dimensional array of doublets for purposes of investigating planar bend propagation incorporating realistic dynein behavior. The contribution of viscous forces to the generation of twist in three-dimensional bending, however, remains to be investigated.

This work was supported in part by National Institutes of Health grant NS 11613.

Received for publication 23 July 1984 and in final form 13 November 1984.

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